SUPPORTING INFORMATION

N-aryl 2-aryloxyacetamides as a new class of fatty acid amide hydrolase (FAAH) inhibitors

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Contents:

1. Docking method

An X-ray crystal structure of fatty acid amide hydrolase (FAAH) from rat in complex with the inhibitor 1-{(3S)-1-[4-(1-benzofuran-2-yl)pyrimidin-2-yl]piperidin-3-yl}-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (PDB code: 3QJ9,[1] resolution 2.3 Å, Chain A) was prepared for molecular docking by the addition of hydrogens, assignment of protonation states (assuming pH 7.4) and tautomers and energy minimization of hydrogens using the OPLS_2005 force field.[2] Low-energy 3D conformations of molecules **4a**–**t** were generated with protonation states assuming pH 7.4. Protein and small molecule preparation was performed in Maestro (version 10.3, Schrödinger, LLC, New York, NY, USA). The ligand binding site in FAAH was prepared by the removal of three water molecules (W806, W851, and W592) to allow for ligands to occupy the whole pocket. A cubic docking region was defined (centered on the crystal ligand with coordinates x=-14.939, y=-1.263, z=-4.692) with dimensions 10*15*10 Å including the entre pocket. Docking was performed in Glide SP (version 6.8, Schrödinger, LLC, New York, NY, USA). A low energy conformation of crystal ligand was docked back to the FAAH protein using the above settings, resulting in a top ranked binding pose with an RMSD of 0.33 Å to the crystal structure conformation, indicating a reliable docking protocol in terms binding pose accuracy. Molecules **4a**–**t**was docked to FAAH with a requirement to match the placement (within a root-mean-square distance (RMSD) of 1.0 Å) of the benzene group of molecules**4a**–**t** to the pyrimidine of the crystal ligand; this requirement was necessary to achieve docking poses similar to the crystal ligand. A maximum of ten binding poses was saved for each molecule during docking. The five top-ranked binding poses of each molecule were rescored using the molecular mechanics generalized Born surface area continuum solvation (MM-GBSA) method[3] implemented in Prime (version 4.1, Schrödinger, LLC, New York, NY, USA). The force field was OPLS_2005[2] and the solvent was implicit water (surface generalized born (SGB) solvent model with dielectric constant equal to 80). Protein residues were static while the docked molecule was allowed to move during the MM-GBSA energy minimization protocol. The binding free energy ΔG_{bind} in kcal/mol was calculated from the difference in energy between the complex and the ligand and protein separately.

Docking results

Docking score values for the five top-ranked molecules according to the Glidescore scoring function[4] are presented in table S1. For **4j** and **4s**, two and three poses, respectively, were generated in total. According to Glidescore the crystal ligand was the highest ranked docked

compound, followed by **4e**, **4f** and **4o**. These three compounds were all *meta*-substituted on the central benzene ring to the oxazolo[4,5-*b*]pyridineor the 1*H*-imidazo[4,5-*b*]pyridine moiety, and these compounds showed inhibition of FAAH. No clear correlation was seen between the Glidescore- and the IC_{50}-values. The crystal ligand was the strongest binder according to MM-GBSA followed by **4h**, **4k**, **4e** and **4q**. According to MM-GBSA, molecules that were *meta*-substituted on the central benzene ring to the oxazolo[4,5-*b*]pyridineor the 1*H*-imidazo[4,5-*b*]pyridine moiety and carrying a 2-hexene moiety would be strong binders. The IC_{50} of these compounds raged from 2.1 to 73 µM and one compound was inactive, indicating that the MM-GBSA energy estimations are limited in terms of their use for IC_{50} predictions.

Table S1. Docking results for molecules **4a**–**t** and the crystal ligand including docking score (Glidescore) and MM-GBSA results, including estimated binding energies (ΔG).

Compound	MM-GBSA ΔG (kcal/mol)	Glidescore	SMILES
4a	-68	-8.1	c1ccnc(c12)nc(o2)-c3c(cccc3)NC(=O)COc(cc4)ccc4-c5ccccc5
	-67	-7.9	
	-59	-7.8	
	-56	-7.8	
	-37	-7.6	
4b	-78	-8.3	c1ccnc(c12)nc(o2)-c3c(cccc3)NC(=O)COc(cc4)ccc4C5CCCCC5
	-73	-8.2	
	-69	-8.2	
	-63	-8.1	
	-73	-7.5	
4c	-78	-7.1	CCC/C=C/COCC(=O)Nc(cccc1)c1-c(o2)nc(c23)nccc3
	-80	-6.8	
	-78	-6.6	
	-81	-6.6	
	-80	-6.6	
4d	-72	-9.3	c1ccccc1OCC(=O)Nc(ccc2)cc2-c(o3)nc(c34)nccc4
	-73	-9.2	
	-73	-9.2	
	-65	-9.2	
	-61	-9.2	
4e	-80	-10.4	CC(C)c1ccc(cc1)OCC(=O)Nc(ccc2)cc2-c(o3)nc(c34)nccc4
	-84	-10.3	
	-80	-10.1	
	-84	-10.1	
	-70	-9.9	
4f	-72	-10.1	c1ccnc(c12)nc(o2)-c3cc(ccc3)NC(=O)COc(cc4)ccc4-c5ccccc5
	-67	-10.0	
	-70	-9.8	
	-70	-9.7	
	-67	-9.7	
4g	-62	-9.5	c1ccnc(c12)nc(o2)-c3cc(ccc3)NC(=O)COc(cc4)ccc4C5CCCCC5
	-60	-8.9	
	-29	-8.8	
	-57	-8.8	
	-56	-8.7	
4h	-83	-9.2	CCC/C=C/COCC(=O)Nc(ccc1)cc1-c(o2)nc(c23)nccc3
	-78	-8.9	
	-81	-8.8	
	-86	-8.8	
	-88	-8.8	

4i	-83	-9.8	c1ccccc1-c2ccc(cc2)OCC(=O)Nc(cc3)ccc3-c(o4)nc(c45)nccc5
	-83	-9.4	
	-78	-8.9	
	-78	-7.7	
	-26	-7.3	
4j	-75	-8.5	C1CCCCC1c2ccc(cc2)OCC(=O)Nc(cc3)ccc3-c(o4)nc(c45)nccc5
	-17	-8.2	
4k	-80	-9.0	CCC/C=C/COCC(=O)Nc(cc1)ccc1-c(o2)nc(c23)nccc3
	-82	-9.0	
	-88	-8.8	
	-80	-8.8	
	-84	-8.8	
4l	-59	-8.0	c1ccnc(c12)nc([nH]2)-c3c(cccc3)NC(=O)COc(cc4)ccc4-c5ccccc5
	-50	-7.6	
	-28	-7.6	
	-29	-7.4	
	-48	-7.2	
4m	-65	-8.0	c1ccnc(c12)nc([nH]2)-c3c(cccc3)NC(=O)COc(cc4)ccc4C5CCCCC5
	-53	-7.6	
	-71	-7.6	
	-65	-7.5	
	-58	-7.4	
4n	-74	-7.5	CCC/C=C/COCC(=O)Nc(cccc1)c1-c([nH]2)nc(c23)nccc3
	-68	-7.0	
	-67	-6.8	
	-67	-6.8	
	-73	-6.8	
4o	-63	-10.1	c1ccnc(c12)nc([nH]2)-c3cc(ccc3)NC(=O)COc(cc4)ccc4-c5ccccc5
	-68	-9.9	
	-68	-9.7	
	-69	-9.6	
	-62	-9.5	
4p	-16	-9.3	c1ccnc(c12)nc([nH]2)-c3cc(ccc3)NC(=O)COc(cc4)ccc4C5CCCCC5
	-59	-9.2	
	-33	-8.9	
	-54	-8.8	
	-49	-8.5	
4q	-83	-9.5	CCC/C=C/COCC(=O)Nc(ccc1)cc1-c([nH]2)nc(c23)nccc3
	-80	-8.8	
	-79	-8.8	
	-84	-8.8	
	-82	-8.7	
4r	-78	-9.8	c1ccccc1-c2ccc(cc2)OCC(=O)Nc(cc3)ccc3-c([nH]4)nc(c45)nccc5
	-78	-9.8	
	-71	-9.4	
	-71	-9.3	
	-78	-9.0	
4s	-71	-9.3	C1CCCCC1c2ccc(cc2)OCC(=O)Nc(cc3)ccc3-c([nH]4)nc(c45)nccc5
	-71	-8.7	
	-63	-8.3	
4t	-71	-8.7	CCC/C=C/COCC(=O)Nc(cc1)ccc1-c([nH]2)nc(c23)nccc3
	-72	-8.6	
	-78	-8.6	
	-80	-8.6	
	-74	-8.6	
Crystal Ligand	-95[a]	-13.2	c1cccc(c12)n(c(=O)n2CC)[C@H]3CCCN(C3)c(n4)nccc4-c(c5)oc(c56)cccc6
		-13.2	
		-13.0	
		-12.9	
		-12.5	

[a]MMGBSA energy value for the conformation of the crystal ligand present in the crystal structure.

Docking references

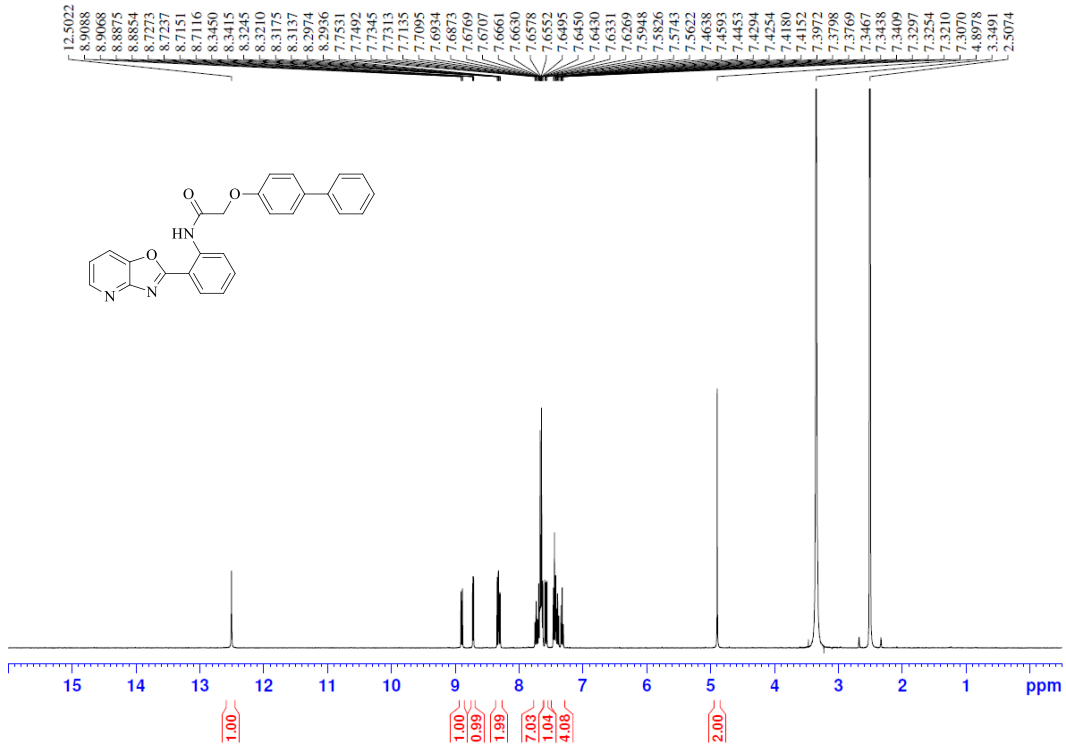
1. Min, X, Thibault ST, Porter AC, et al. Discovery and molecular basis of potent noncovalent inhibitors of fatty acid amide hydrolase (FAAH). Proc Natl Acad Sci USA 2011; 108: 7379–84.
2. Kaminski GA, Friesner RA, Tirado-Rives J, et al.Evaluation and reparametrization of the OPLS-AA force field for proteins via comparison with accurate quantum chemical calculations on peptides. J Phys Chem B 2001; 105: 6474–87.
3. Still WC, Tempczyk A, Hawley RC, et al. Semianalytical treatment of solvation for molecular mechanics and dynamics. J Am Chem Soc 1990; 112: 6127–29.
4. Friesner RA, Banks JL, Murphy RB, et al. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking Accuracy. J Med Chem 2004; 47: 1739–49.

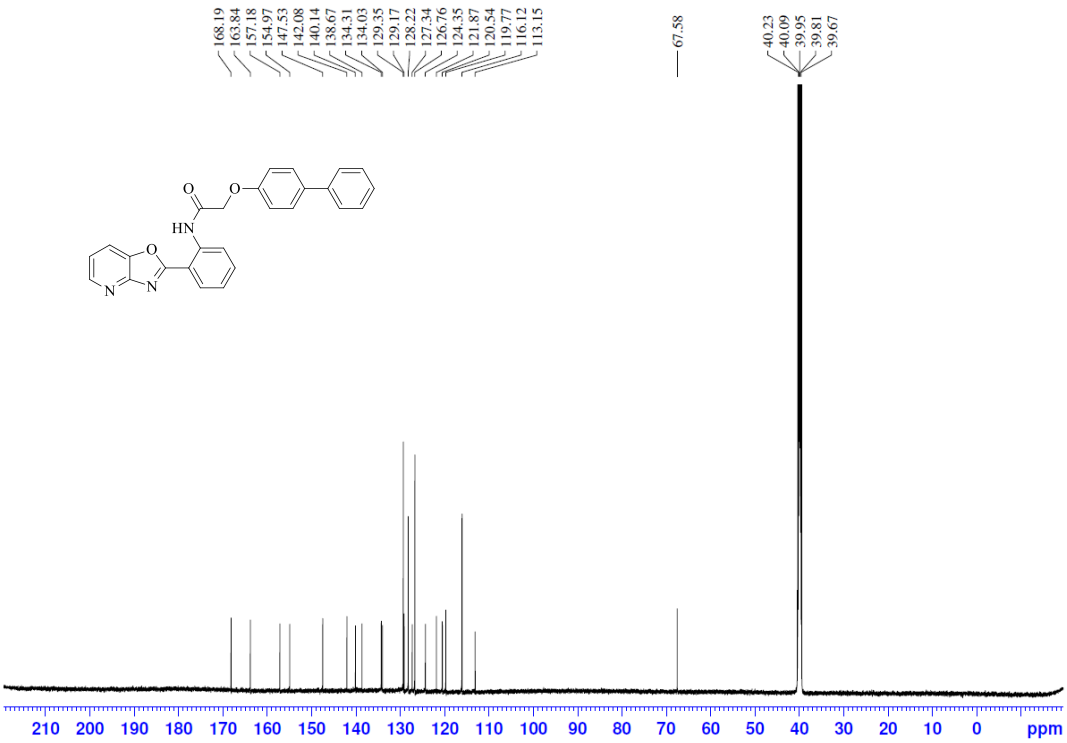
2. Experimental

General

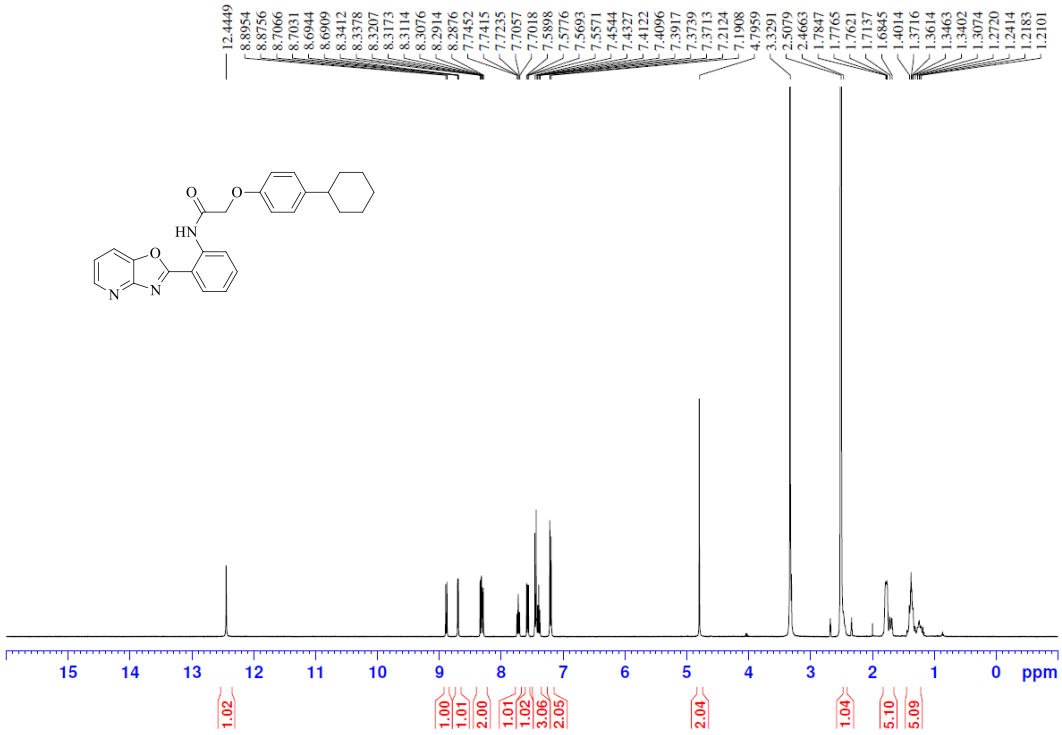
All reagents and solvents were used as received from commercial suppliers, unless indicated otherwise. Compound **3a** was procured from Sigma Aldrich. DMF and THF were dried in a solvent drying system (drying agent: neutral alumina) and collected fresh prior to every reaction. Pyridine and 1,4-dioxane were dried over 4 Å molecular sieves. TLC was performed on alumina-backed silica gel plates (median pore size 60 Å, fluorescent indicator 254 nm) and detected with UV light at 254 nM. Column chromatography was performed using silica gel with an average particle diameter 50 μm (range 40−65 μm, pore diameter 53Å).LC-MS was carried out with a Waters LC system equipped with an Xterra C18 column (50 × 19 mm, 5 μm, 125 Å), eluted with a linear gradient of acetonitrile in water, both of which contained formic acid (0.2%). A flow rate of 1.5 mL/min was used and detection was performed at 254 nm. Mass spectra were obtained on a Water micromass ZQ 2000 using electrospray and detection of positive and negative ions. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Brüker DRX-400 or Brüker DRX-600spectrometer. NMR experiments were conducted in DMSO-d_6 and $CDCl_3$. All final compounds were ≥95% pure according to ^{1}H NMR and LCMS data.

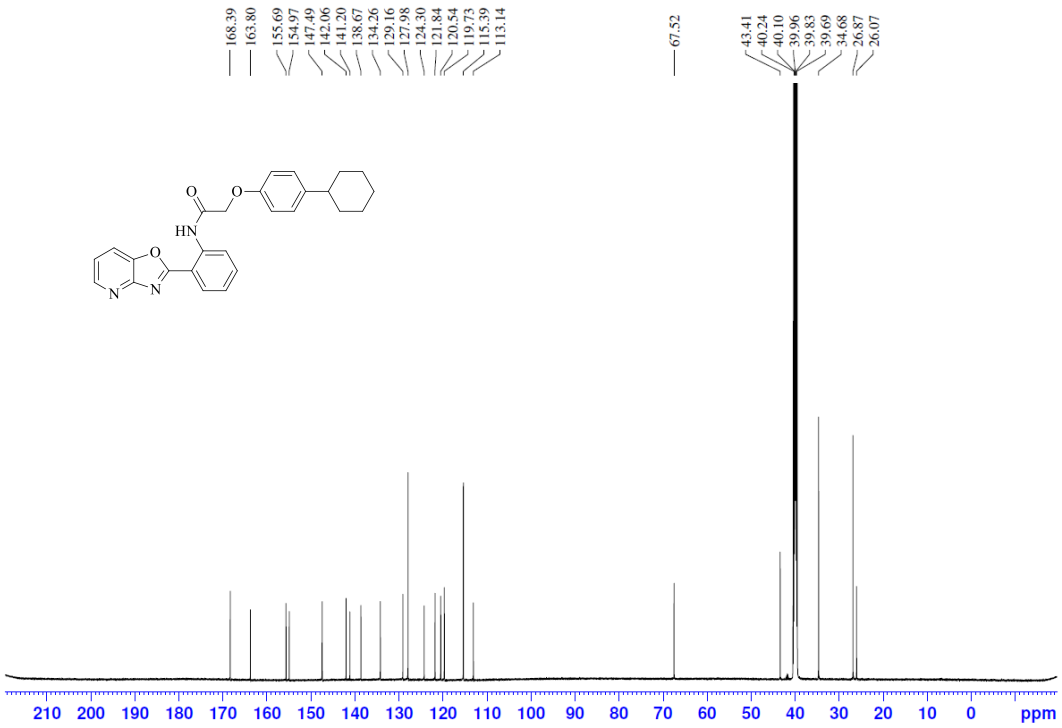
^{1}H and ^{13}C spectra of **4a**





^{1}H and ^{13}C spectra of **4b**





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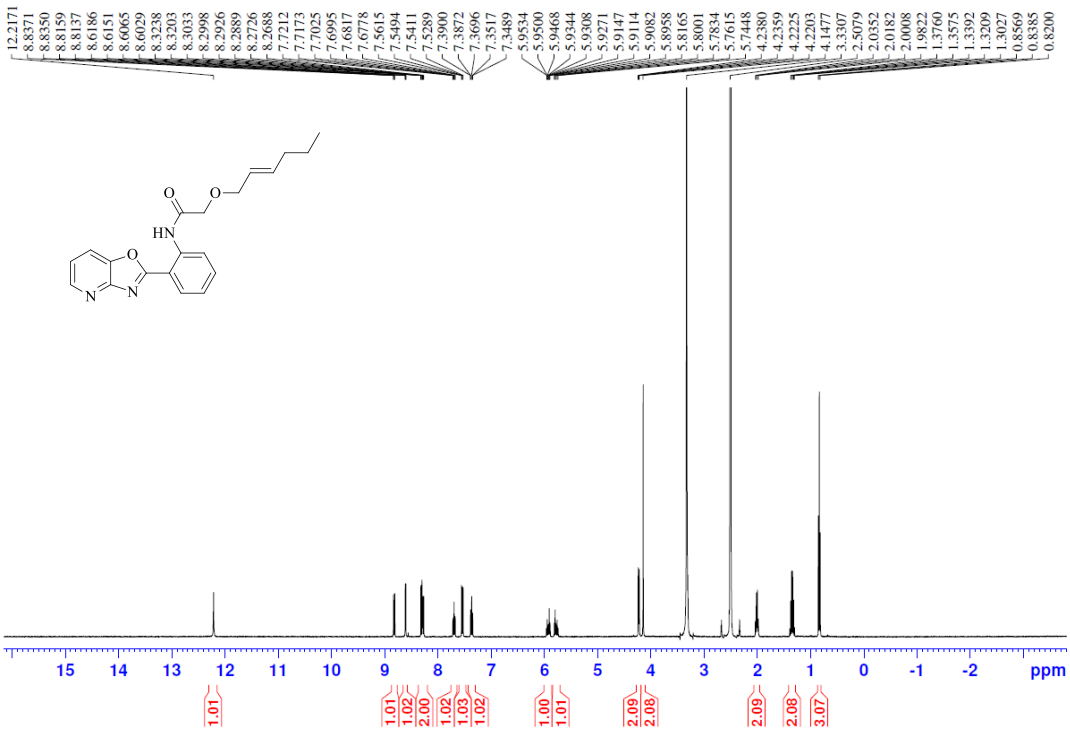

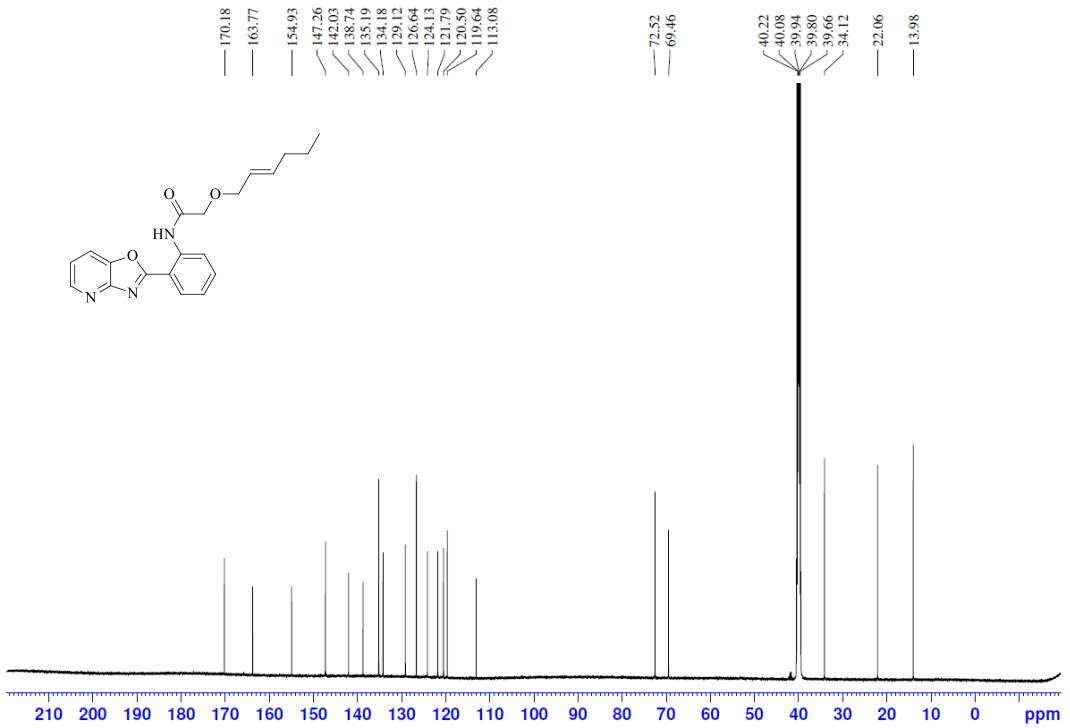

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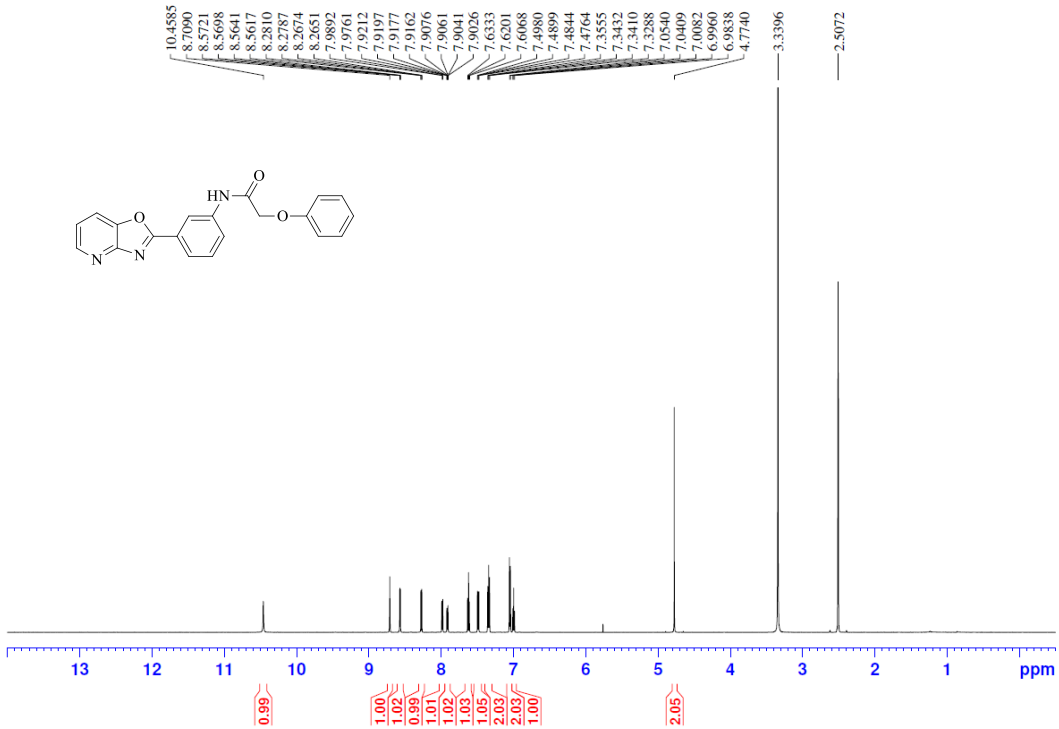

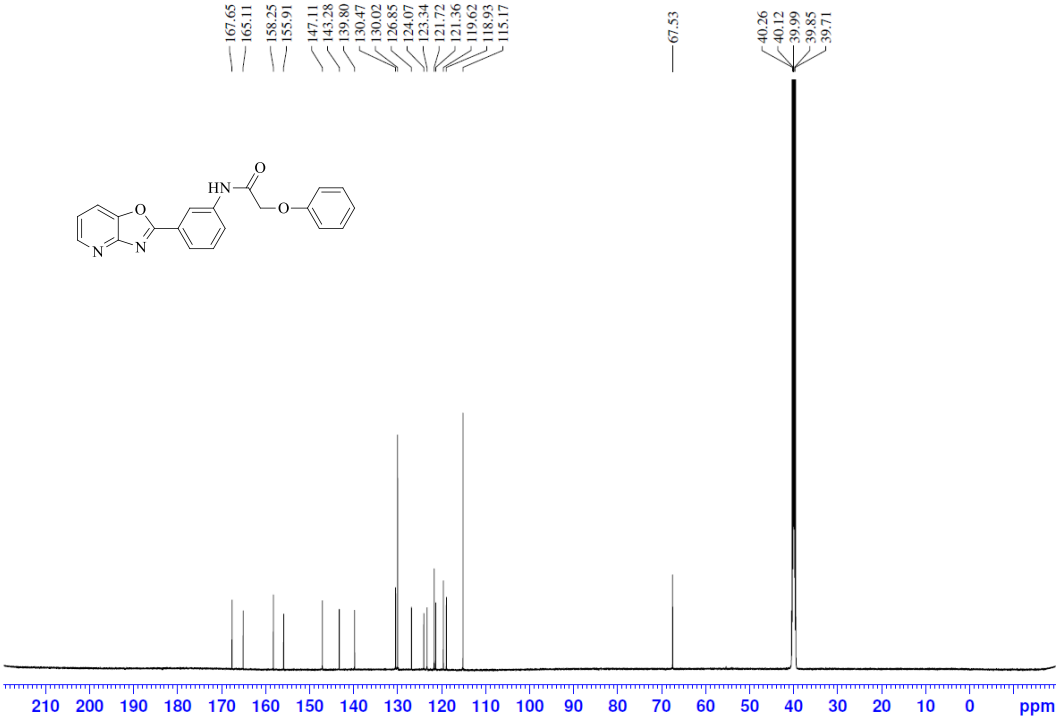

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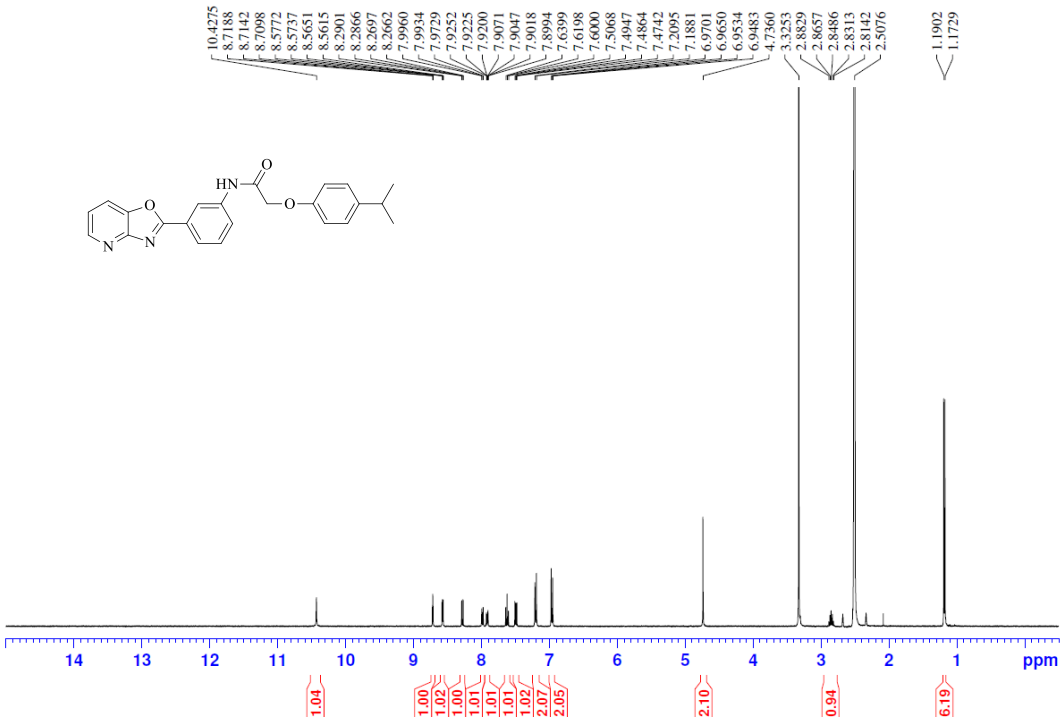

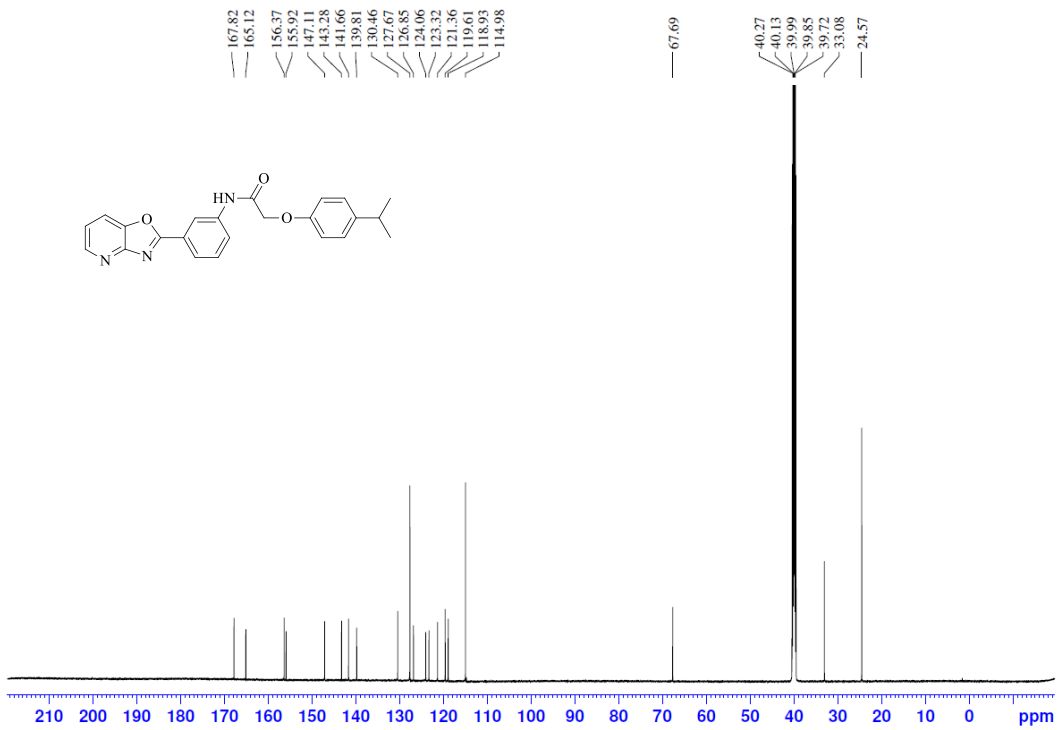

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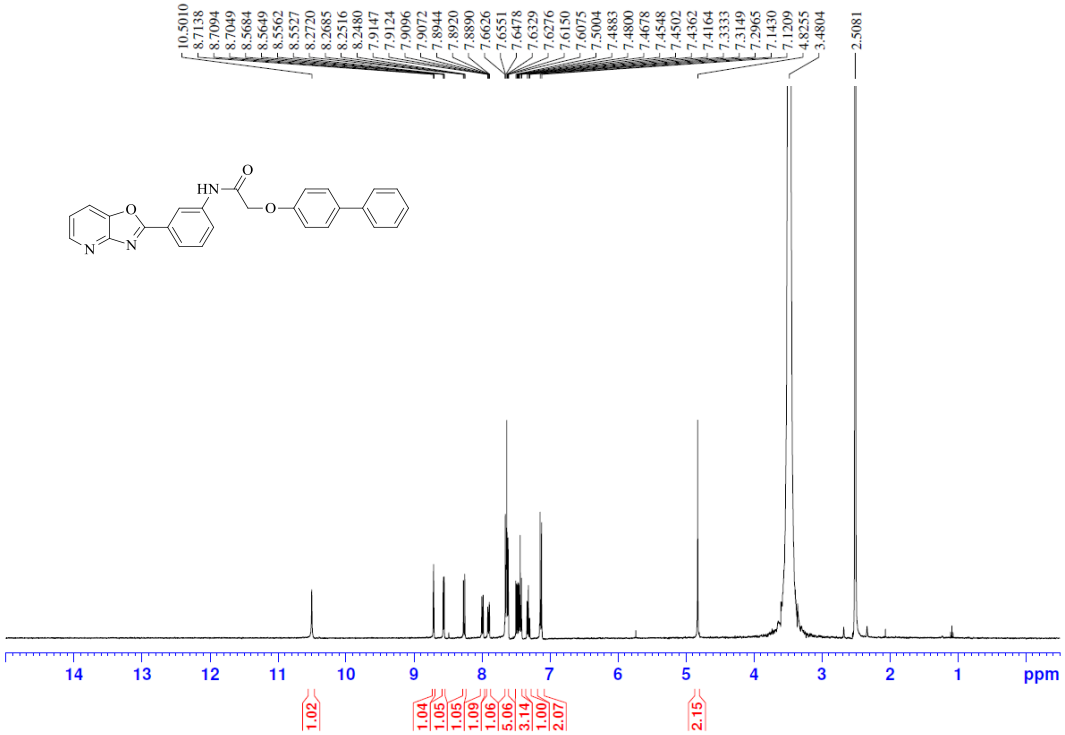

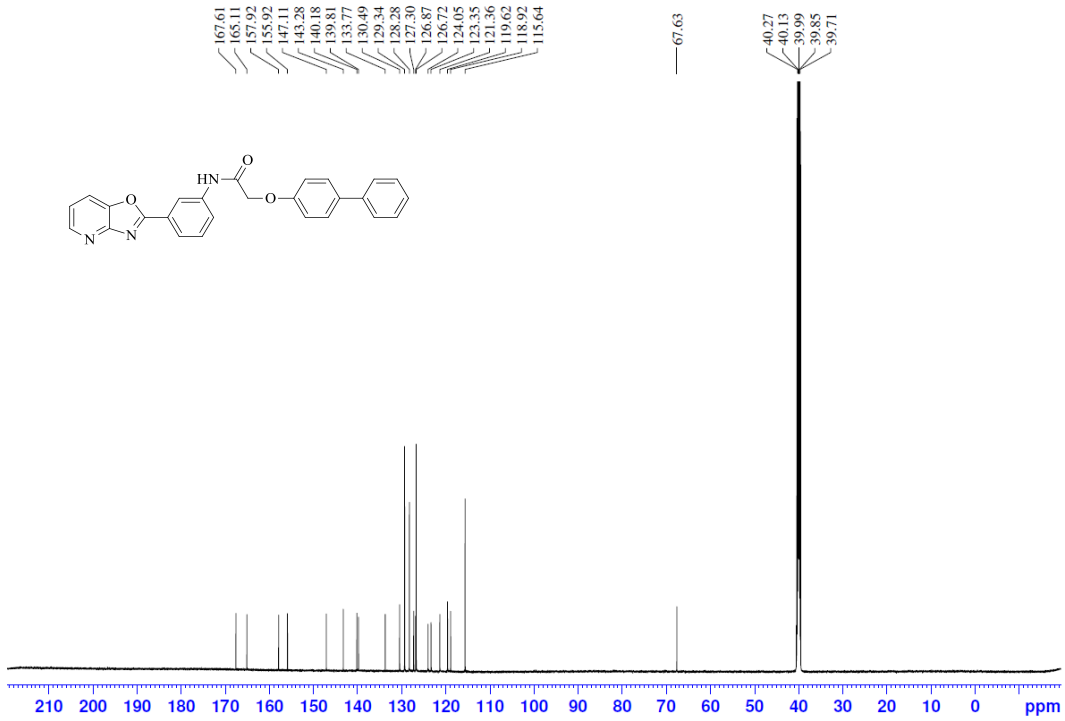

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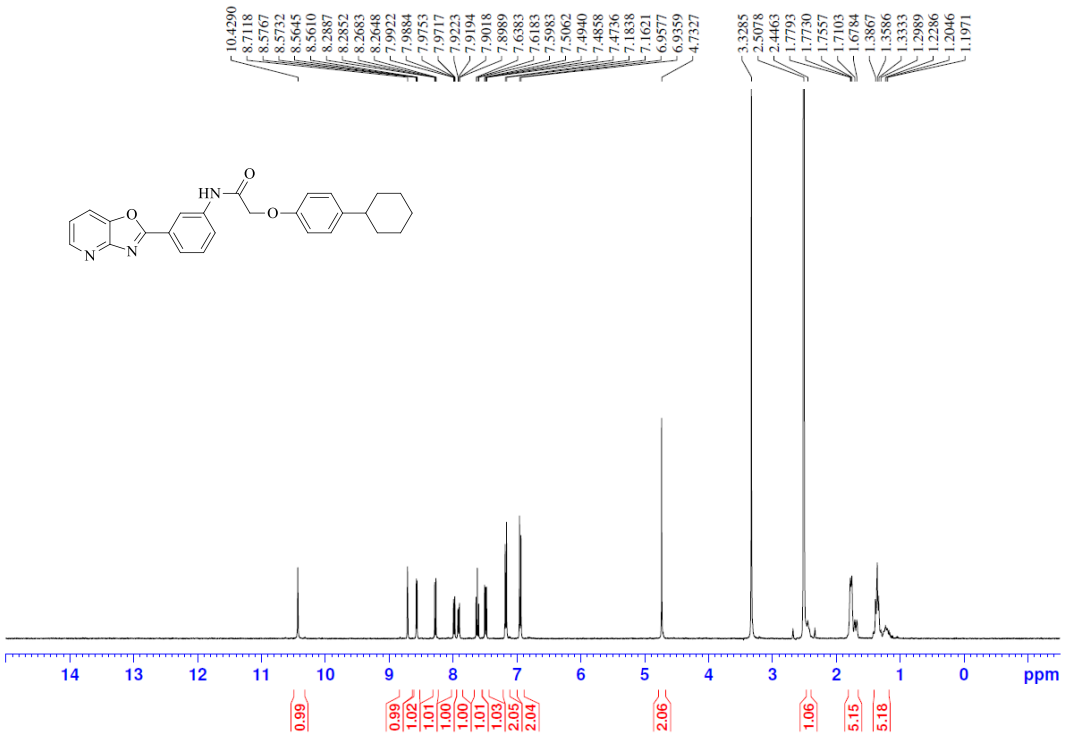

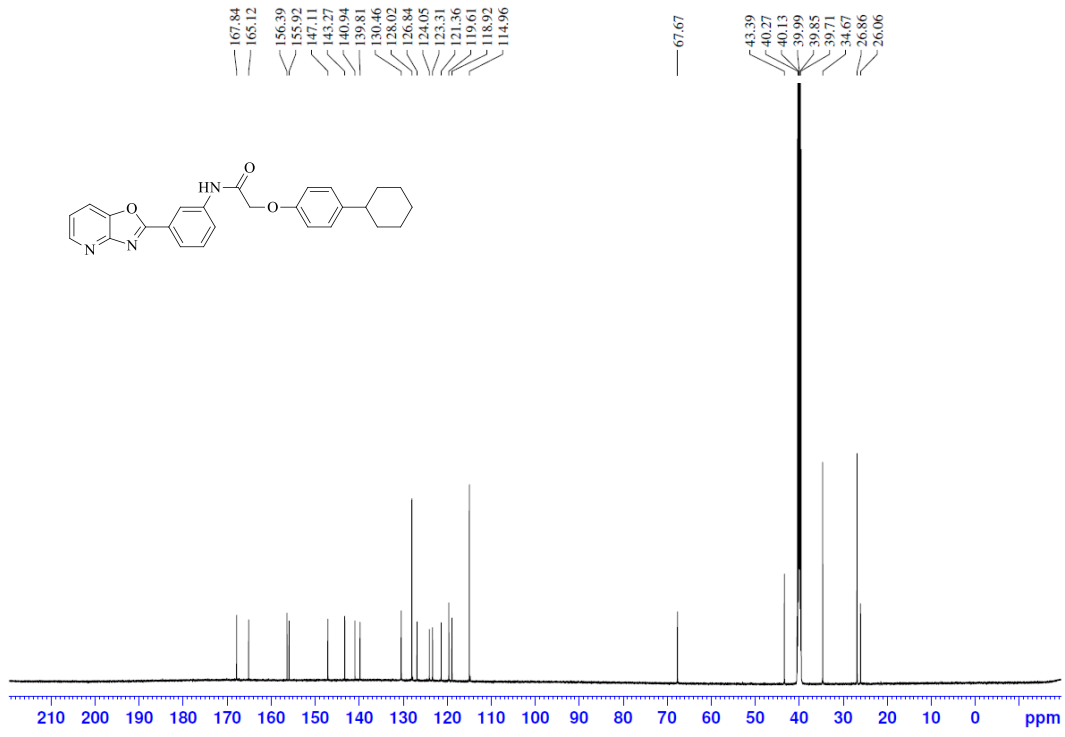

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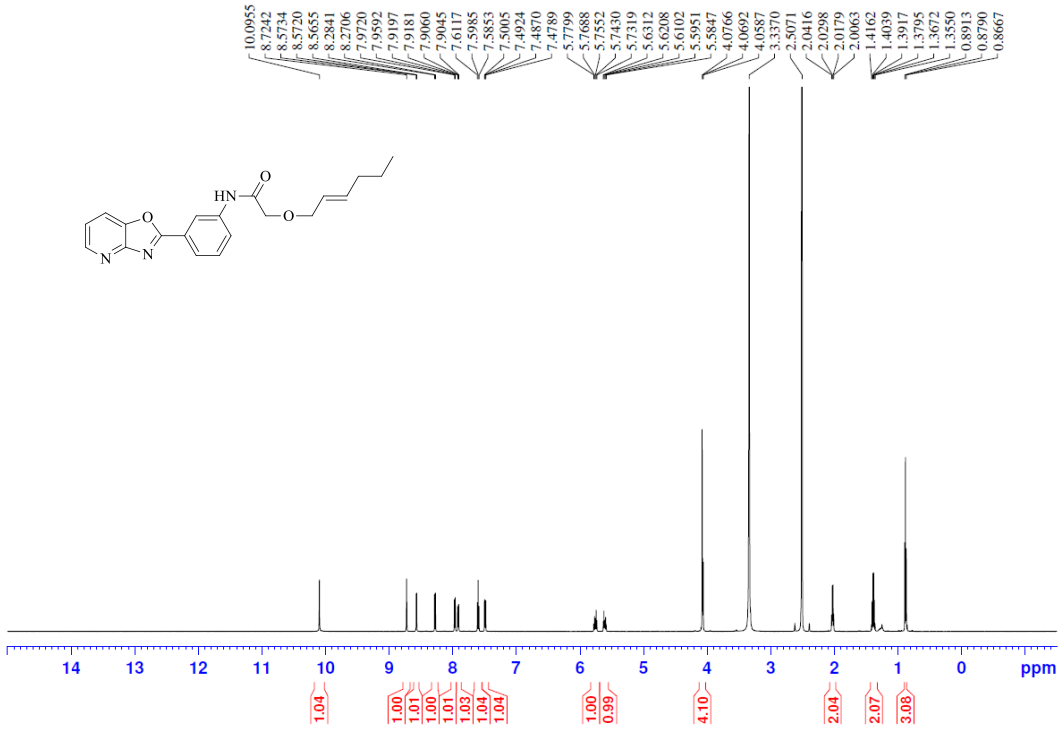

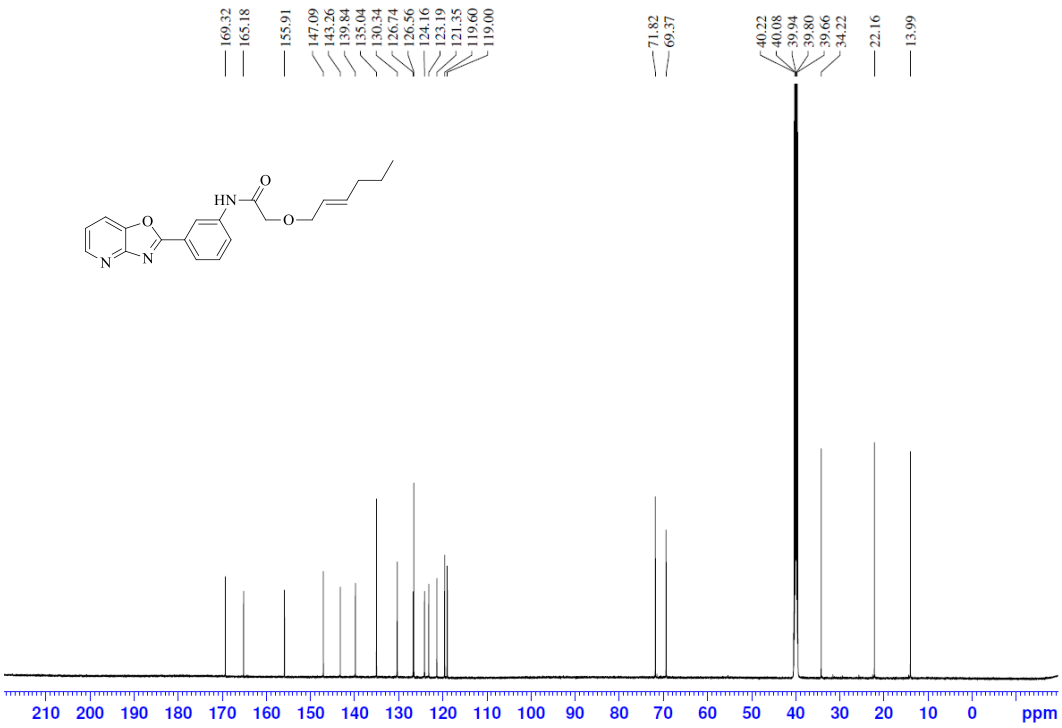

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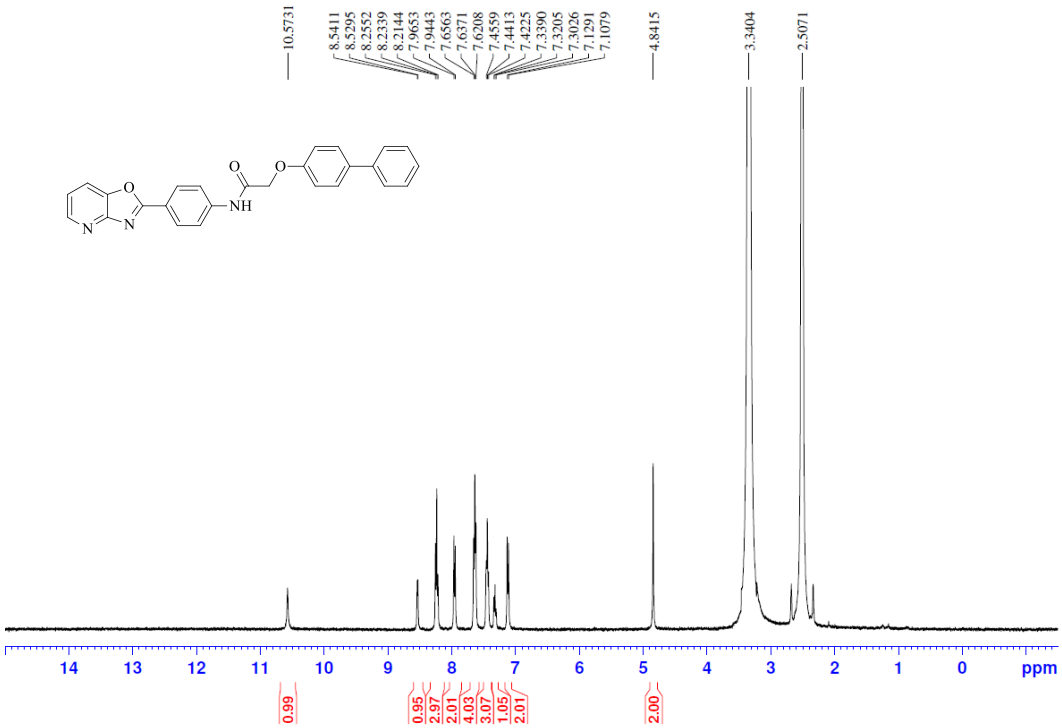

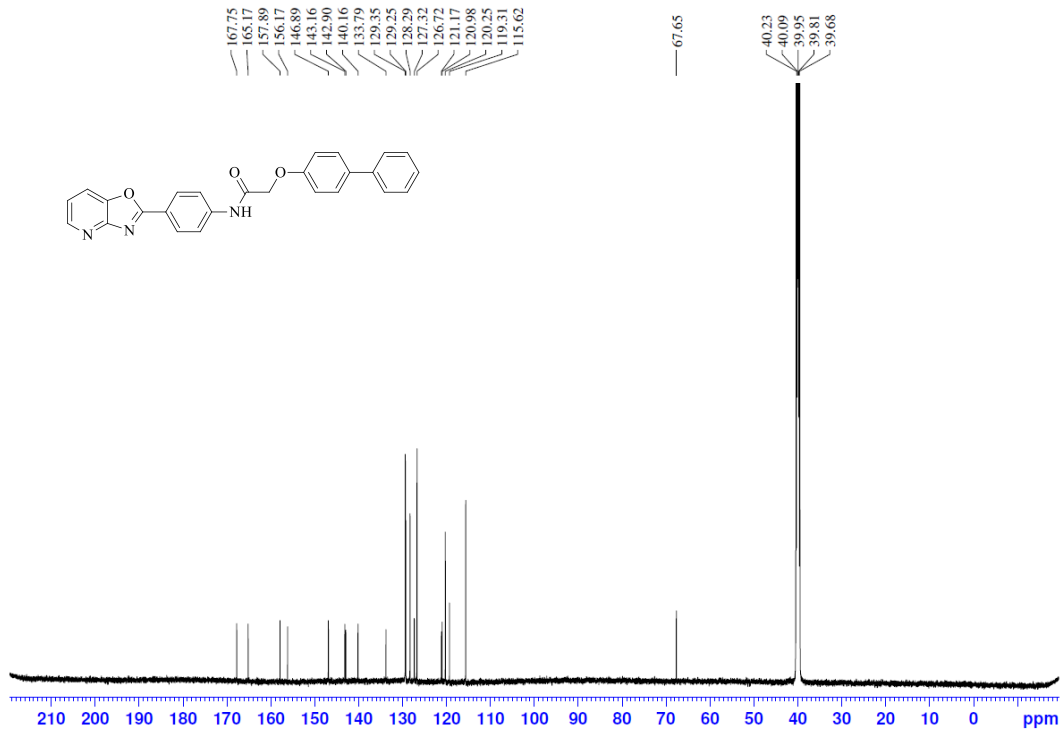

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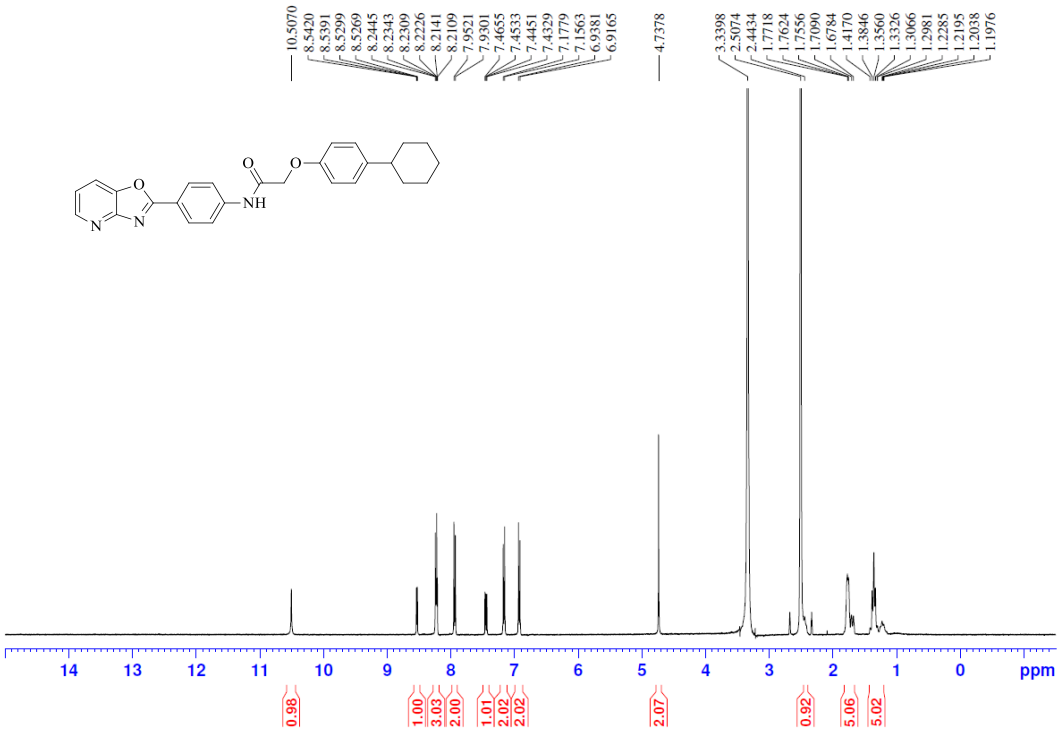

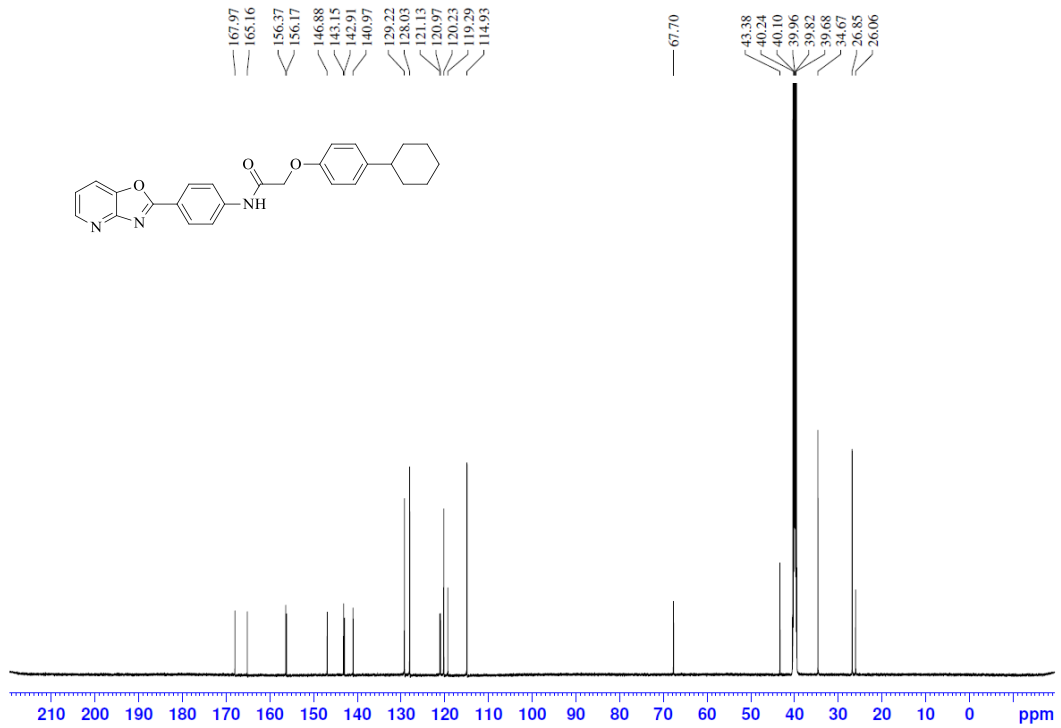

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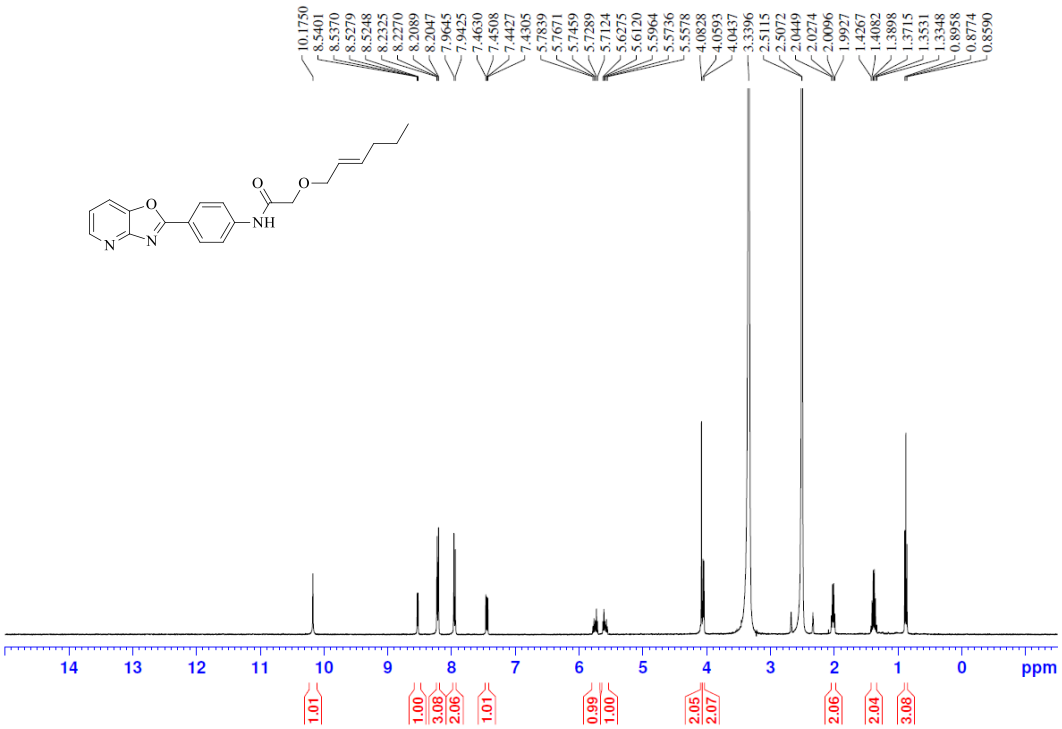

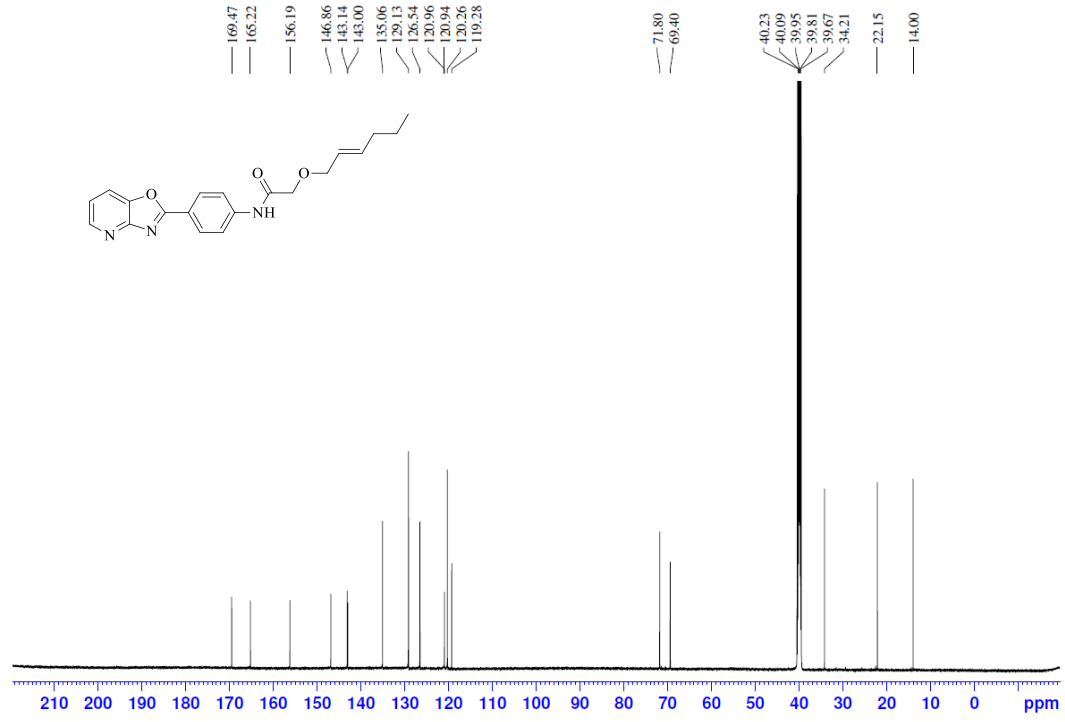

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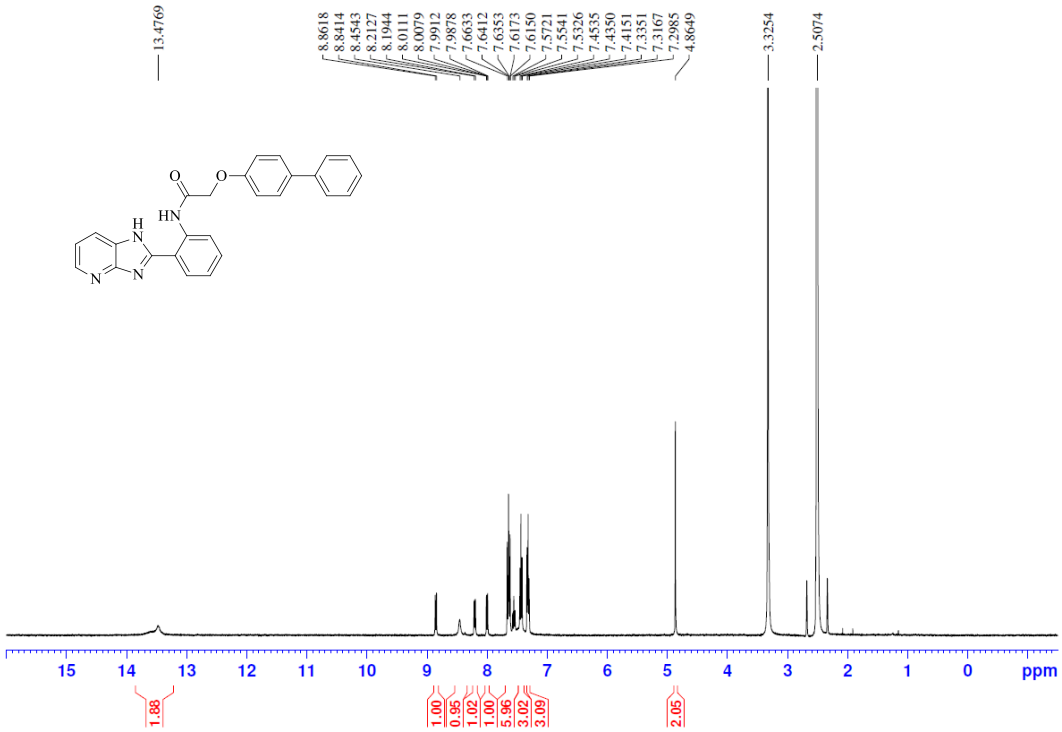
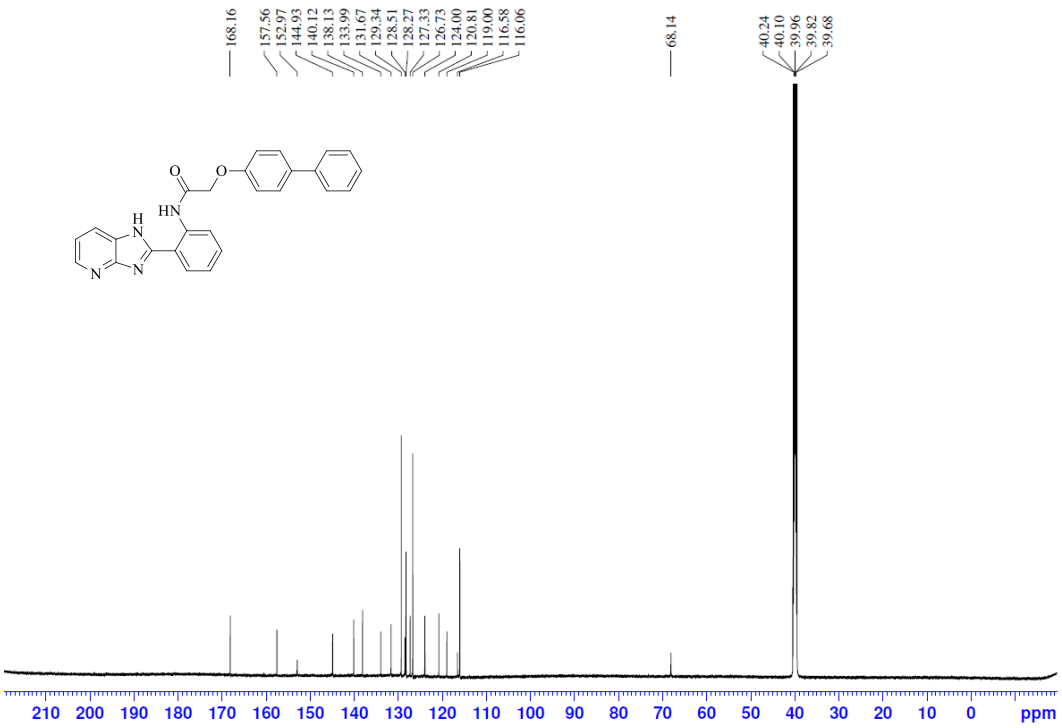
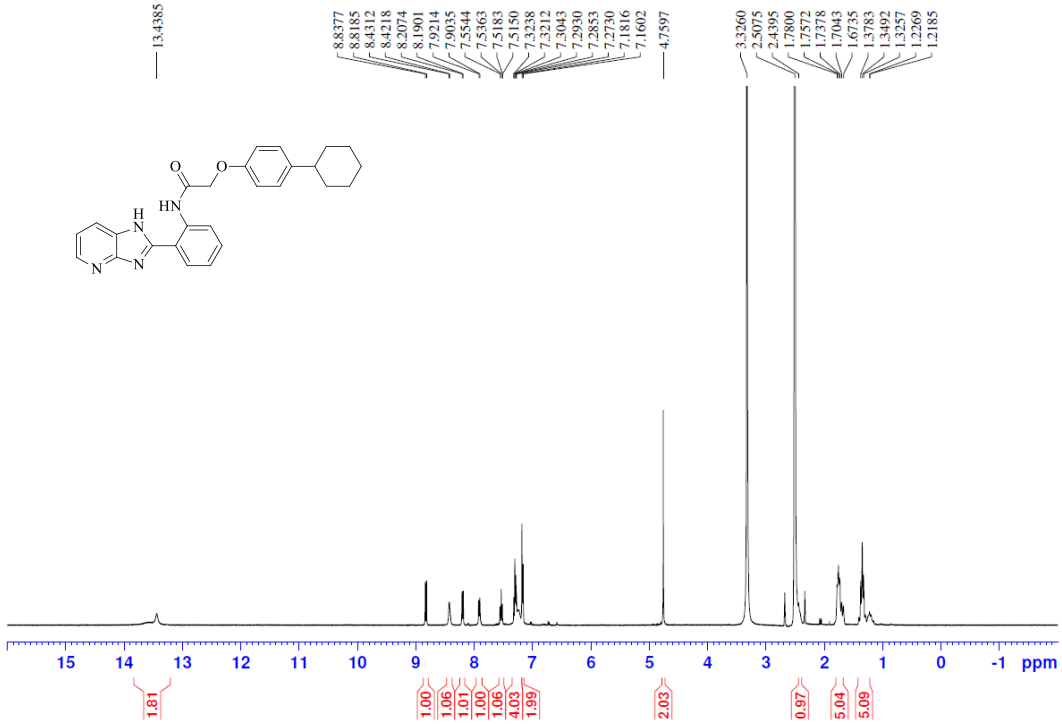
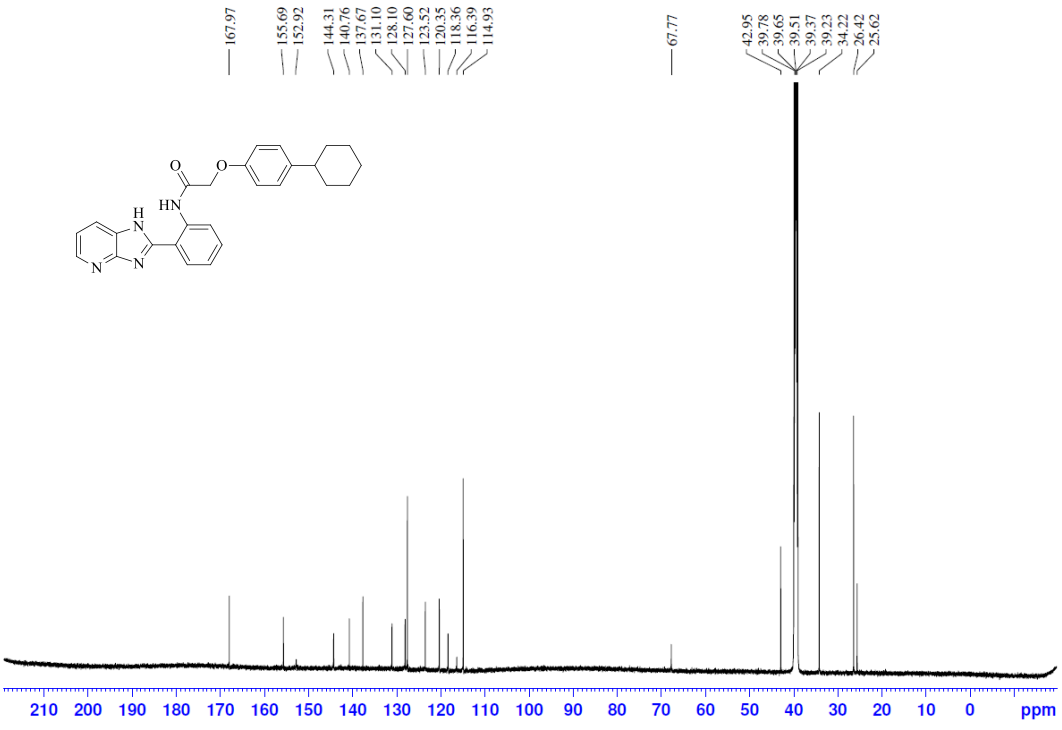




^{1}H and ^{13}C spectra of **4m**





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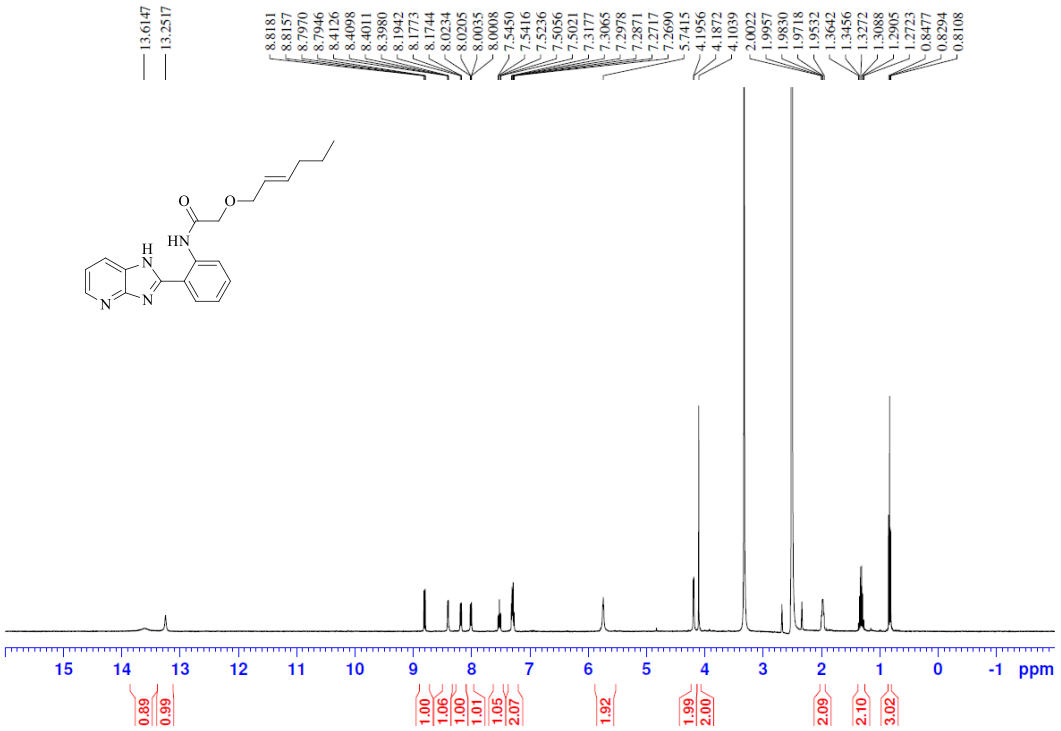

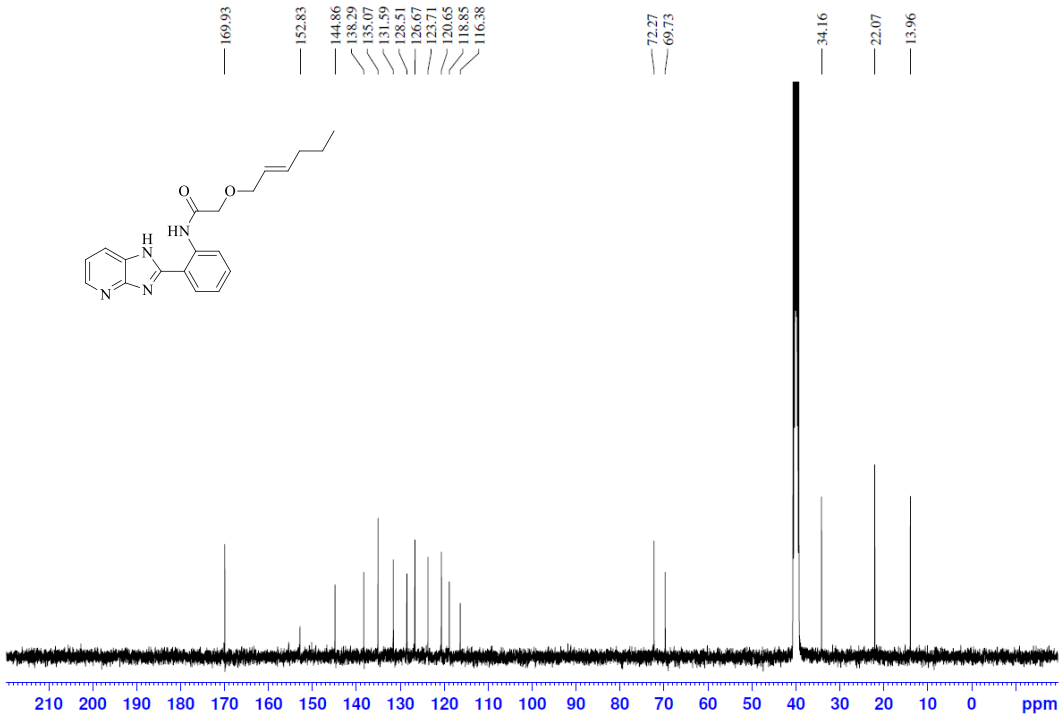

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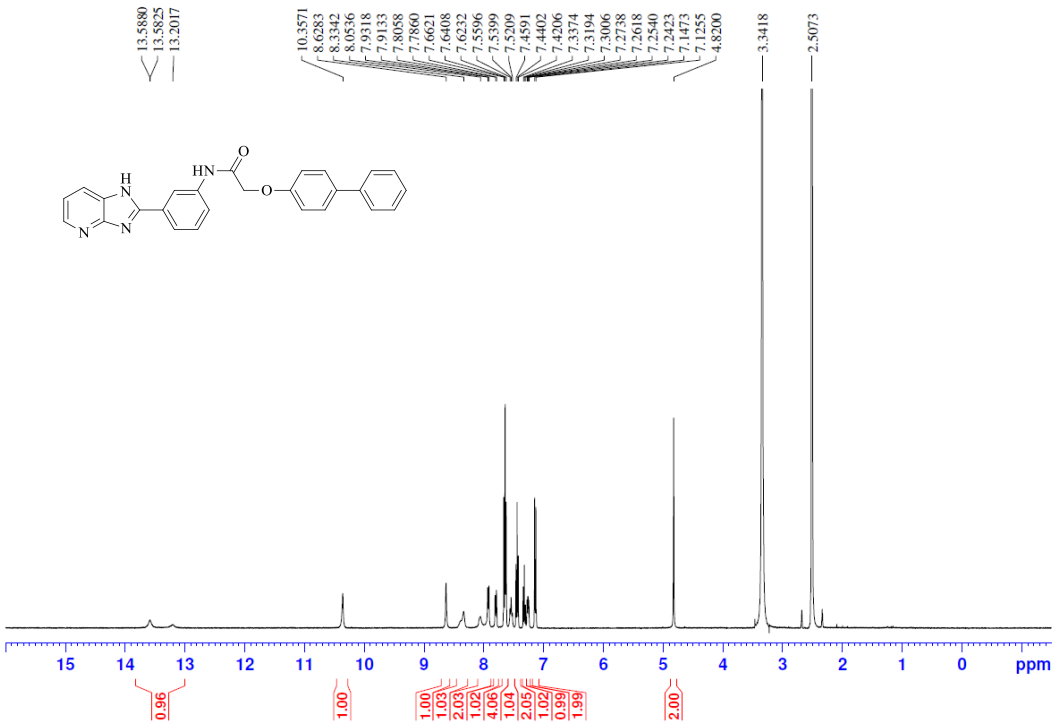
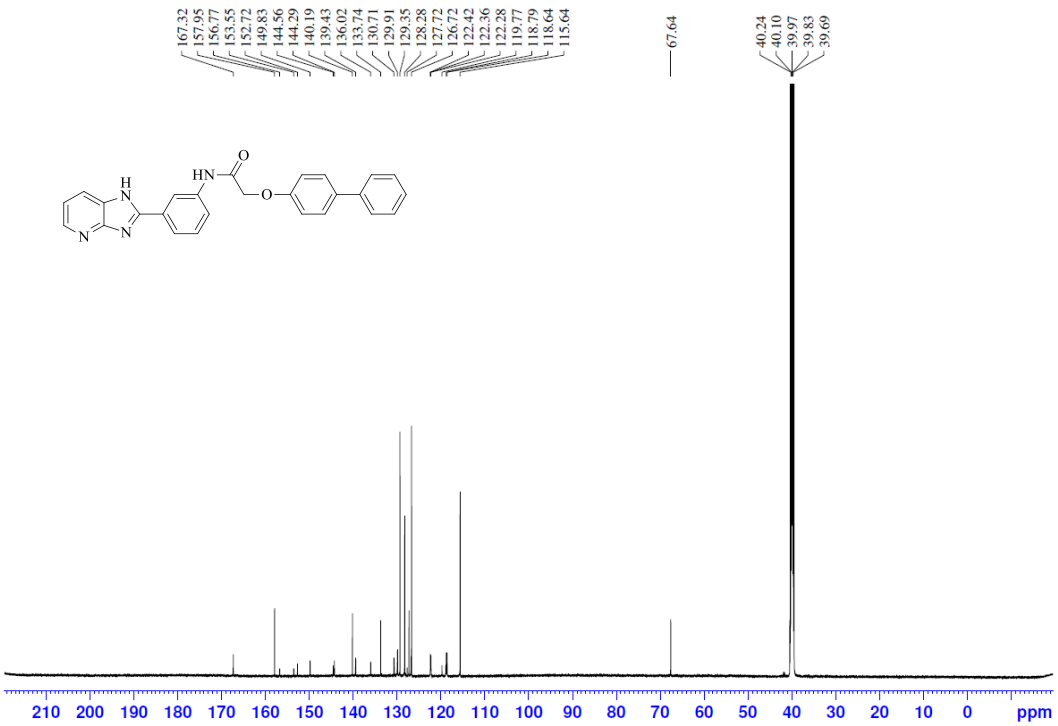
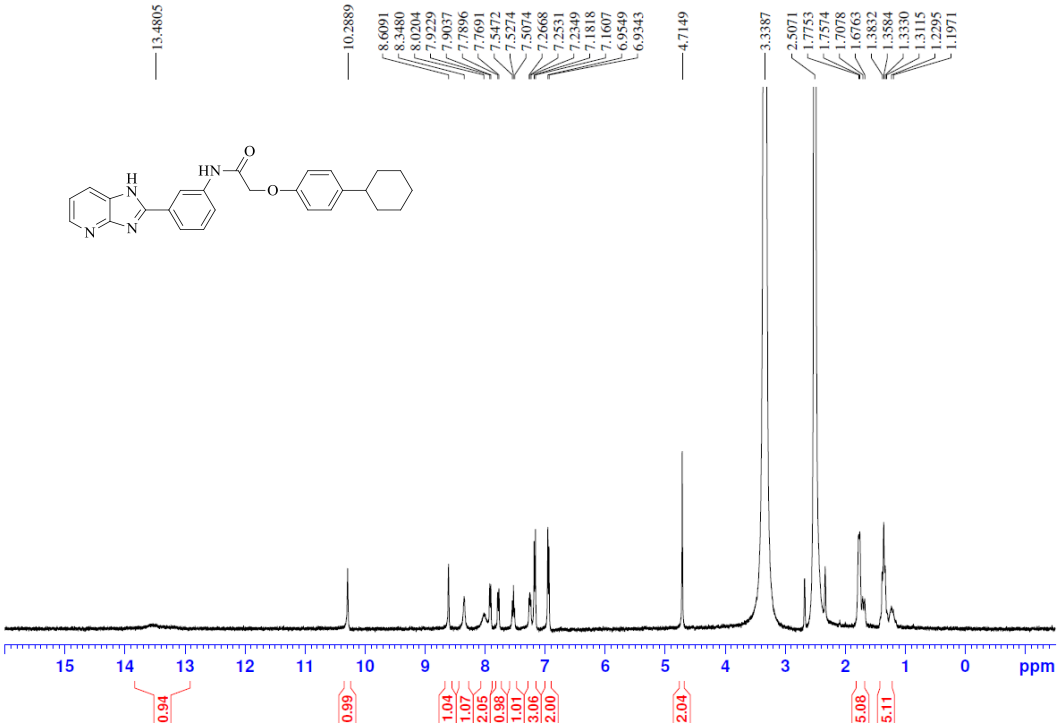
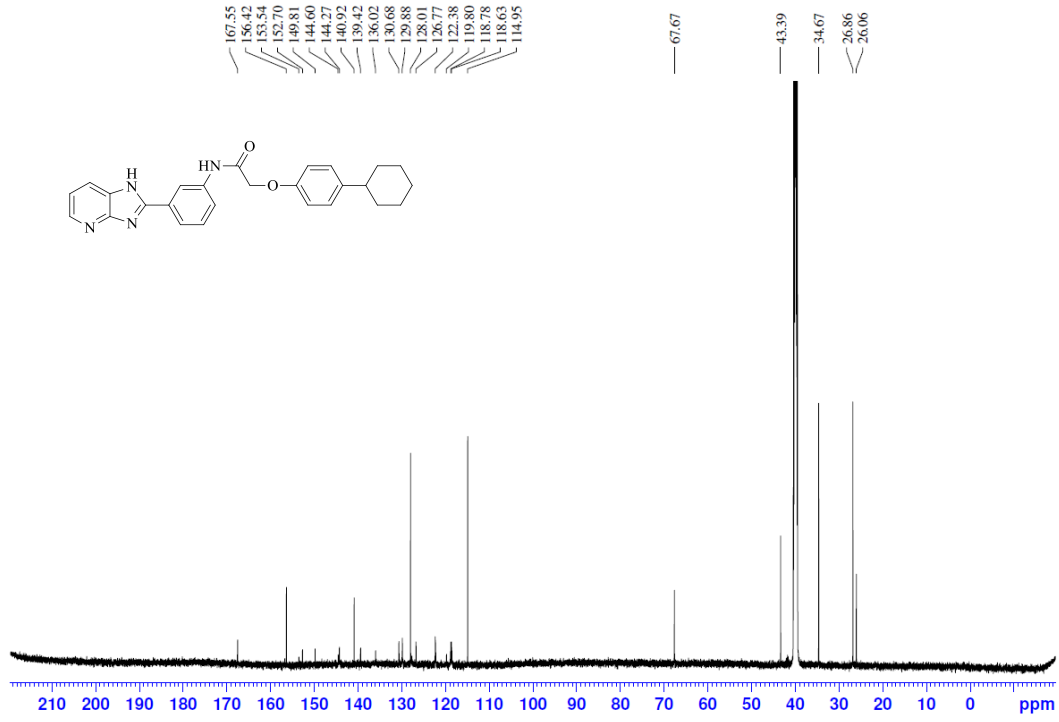


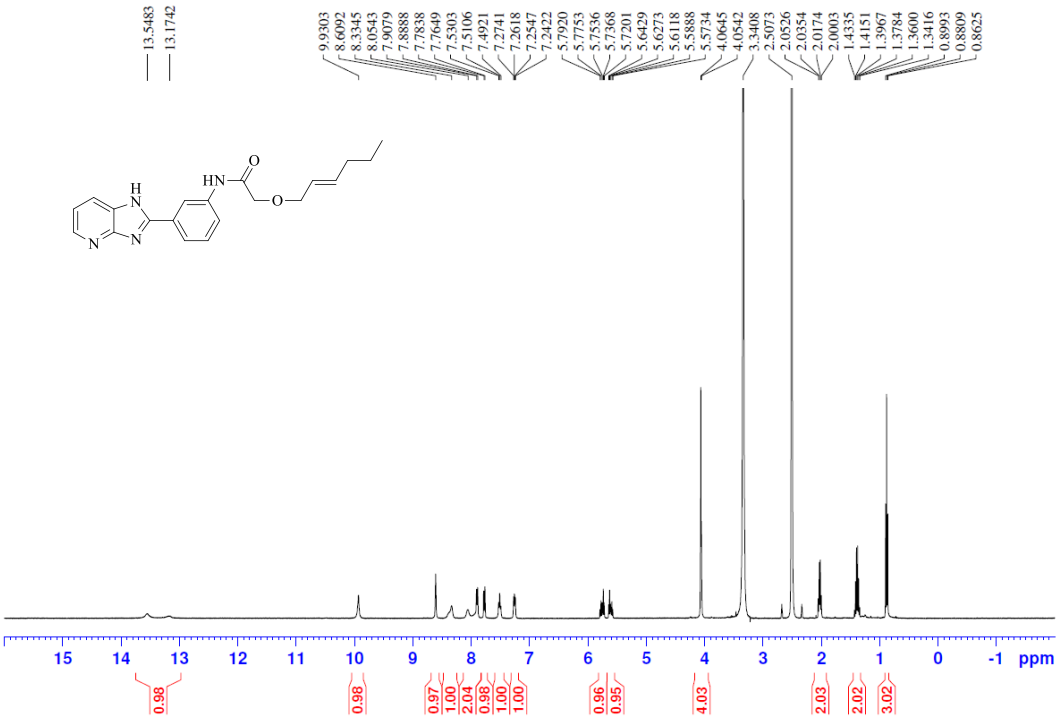


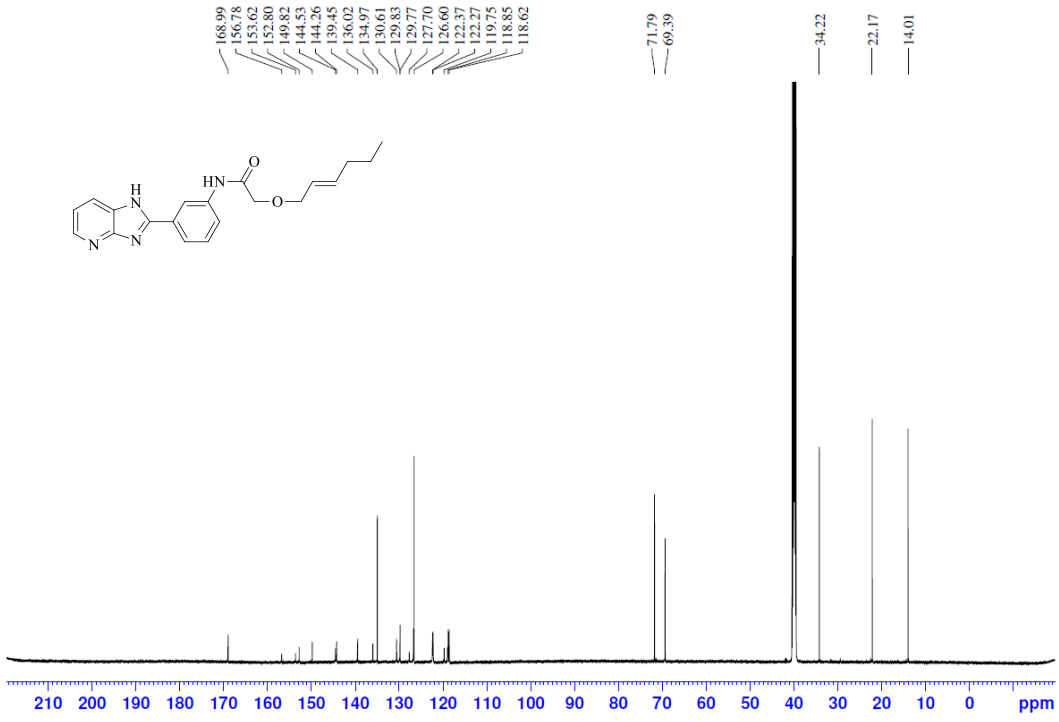
^{1}H and ^{13}C spectra of **4p**



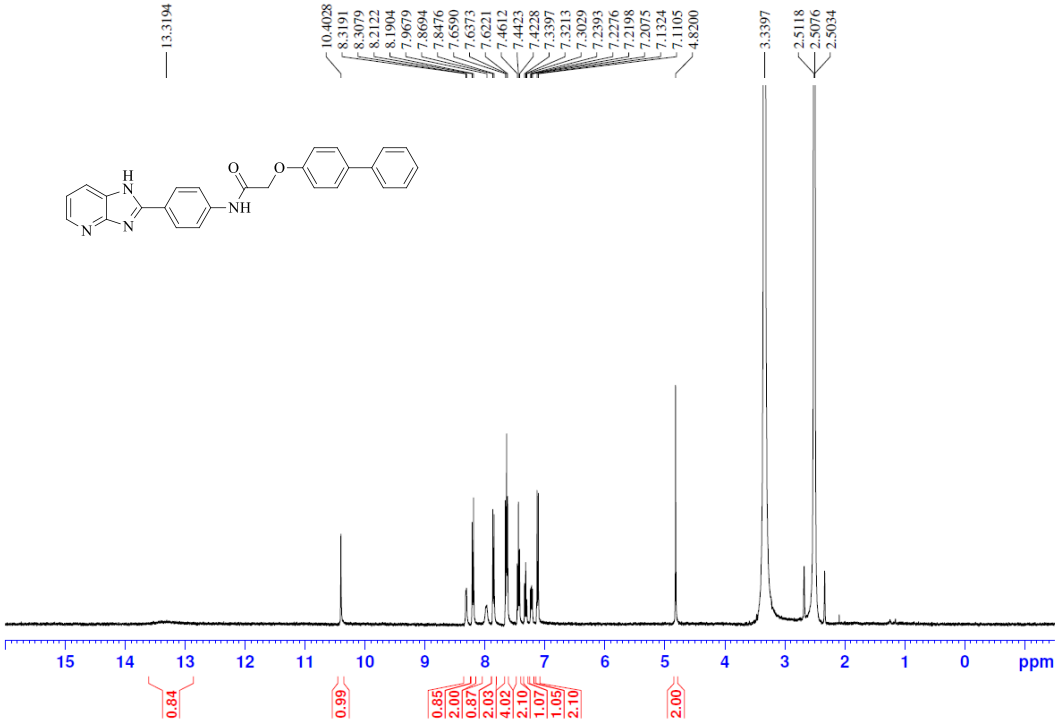


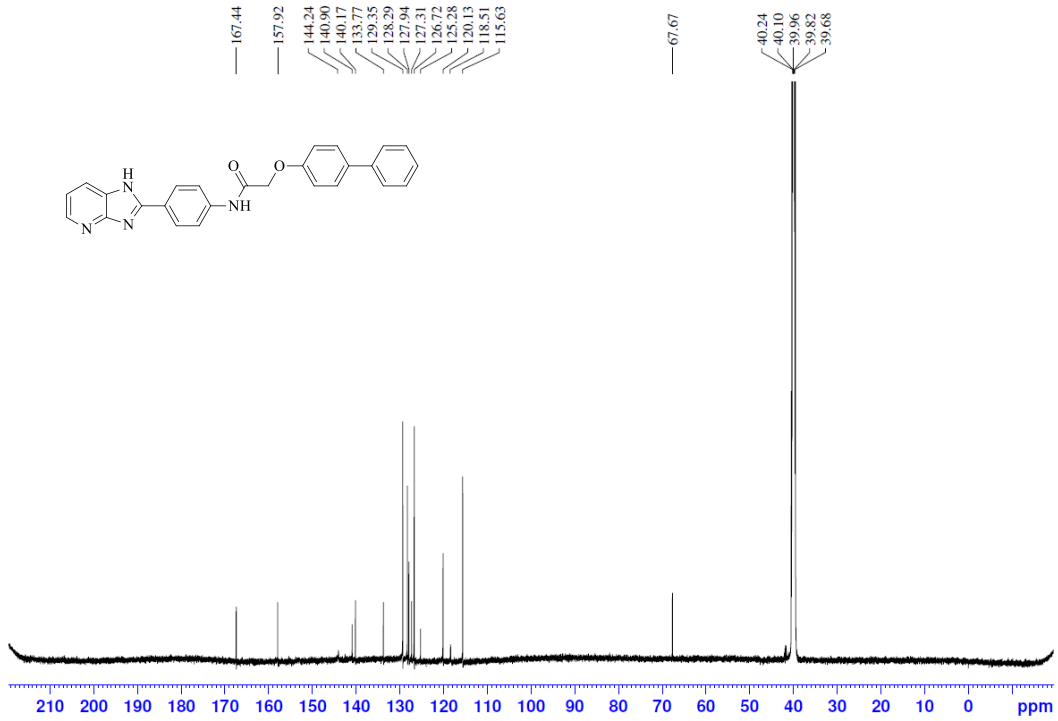
^{1}H and ^{13}C spectra of **4q**



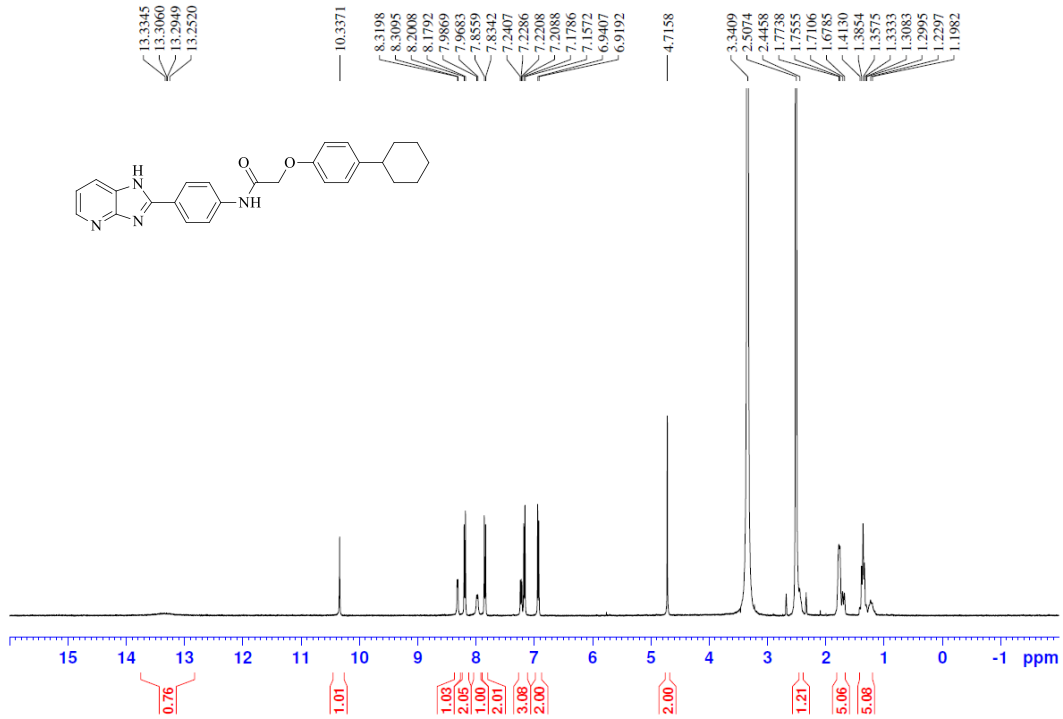


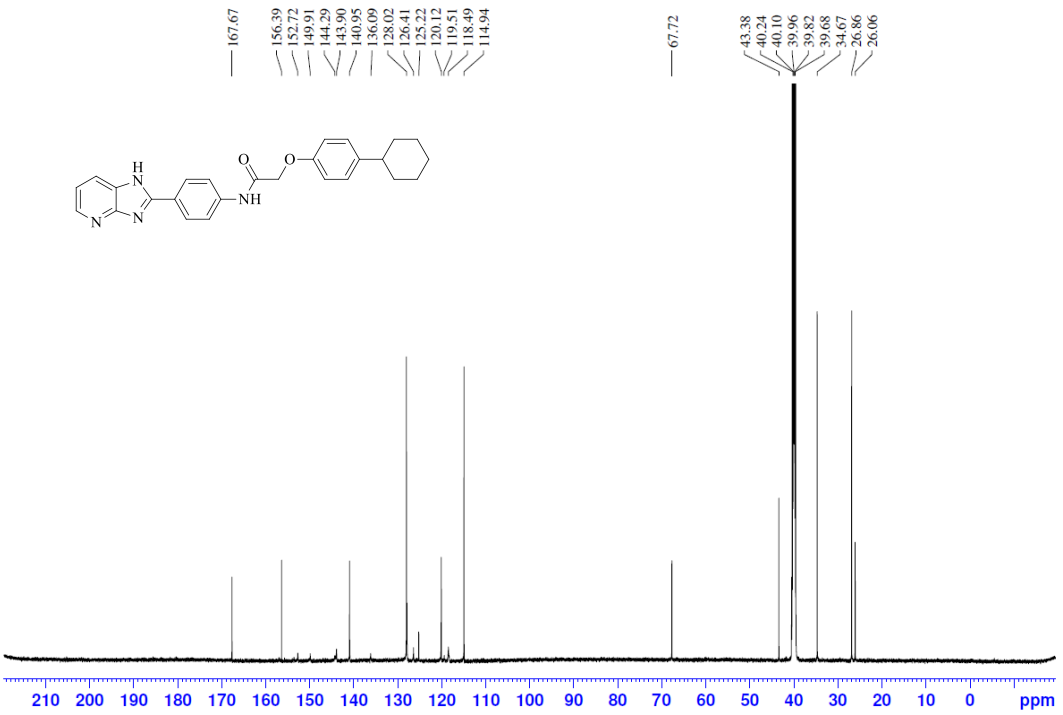
^{1}H and ^{13}C spectra of **4r**





^{1}H and ^{13}C spectra of **4s**





^{1}H and ^{13}C spectra of **4t**